

Optimization of Extraction Process of Carob Bean Gum Purified from Carob Seeds by Response Surface Methodology

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Abstract

The carob product most widely used, especially for the food industry, is the carob bean gum (CBG), or locust bean gum (LBG). This gum comes from the endosperm of the seed and chemically is a polysaccharide, a galactomannan. It is used as thickener, stabilizer, emulsifier and gelling agent.

Response surface methodology (RSM) was applied to optimize the extraction of CBG from Moroccan carob seeds. A central composite design was used for experimental design and analysis of the results searching for the optimal extraction conditions: Extraction temperature, extraction time and water to endosperm seeds ratio. Based on the RSM analysis, optimum conditions were: temperature 97°C, time 36 min and water to endosperm of seeds ratio of (197:1). Under the optimized conditions, the experimental values were in close agreement with values predicted by the model and for wish. Predicted yield of carob gum extracted is 69% of endosperm seeds.

Keywords: Carob bean gum; Extraction; Central composite design; Optimization experiment.

1. Introduction

Carob (*Ceratonia siliqua* L.) is a typical tree of the semiarid environments in the Mediterranean area. This species belongs to the subfamily Caesalpinioideae of the Leguminosae family (Biner et al. 2007). It produces edible pods used as a fodder for breeding cattle; it has also a long history of application as a source of health products. World production is estimated at about 315 000 tons per year, produced from about 200 000 hectares with very variable yields depending on the cultivar, region, and farming practices (Makris & Kefalas 2004) and the main producers for (pulp, seeds) respectively are Spain (36%, 28%), Morocco (24%, 38%), Italy (10%, 8%), Portugal (10%, 8%), Greece (8%, 6%), Turkey (4%, 6%) and Cyprus (3%, 2%) (Ait chitt et al. 2007).

The two main carob pod constituents are pulp (90%) and seeds (10%) by weight (Tous et al. 1995). Carob pulp is high (48–56%) in total sugar content that include mainly sucrose, glucose, fructose and maltose. In addition it contains about 18% cellulose and hemicelluloses, (3–4%) protein and (0.4–0.8%) lipids (Santos et al. 2005). Also, ripe carob pods contain a large amount of condensed tannins (16–20%, d.b.). The pulp of carob pods is used extensively as a raw material for the production of syrups (Petit & Pinilla 1995; El Batal et al. 2011; El Batal et al. 2013) and crystallized sucrose for the food industry. On the other hand, carob seed constituents are seed coat (23–33%), endosperm (42–56%) and embryo (20–25%) by weight (Dakia et al. 2008).

Carob bean gum (CBG), is the refined endosperm of the seed of the carob pods (*Ceratonia siliqua*) by extraction of the seeds with water or aqueous alkaline solutions. The extraction of the gum from the seeds is a slow, difficult process, due principally to the hardness of the seed coat. Many bibliographical studies showed the effect of the conditions of extraction on the yield of extracted polysaccharides (XuJie & Wei 2008; RenJie 2008; Qiao et al. 2009; Firatligil-Durmus & Evranuz 2010).

CBG is a galactomannan composed of a linear chain 1→4 linked β -D-mannopyranosyl units, with α -D-galactopyranosyl residues 1→6 joined as irregularly spaced side chain (Belitz & Grosch 1999).

Featuring different physicochemical properties, CBG is a versatile material used for many applications: they are excellent stiffeners and stabilizers of emulsions, and the absence of toxicity allows their use in the textile, pharmaceutical, biomedical, cosmetics, nutrition sciences, and food industries (Srivastava & Kapoor 2005; Vieira et al. 2007; Matthausa & Ozcanb 2011; Vilà et al. 2012; Karababaa & Coskunerb 2013).

An important application of this biopolymer is its ability to form very viscous solution at relatively low concentration, to stabilize dispersion and emulsion and to replace fat in many dairy products. Carob gum properties are generally unaffected by pH, salts, or heat processing because it is non-ionic (Pollard et al. 2010; El Batal et al. 2011). It is also compatible with other gums and thickening agents (carraghenan, agar, xanthan) to form a more elastic and stronger gel (Puhan & Wielinga 1996). These properties of CBG allow its use as interesting additives for several industries, in particular for the food industry.

The objective of the present work was to optimize and study the effect of extraction temperature, extraction time and water to endosperm seeds ratio on the aqueous extraction yield of gum polysaccharide from carob seeds using the response surface methodology (RSM) widely applied in the food industry to determine the effects of several variables and optimize conditions.

2. Materials and methods

2.1. Samples

Samples of carob were collected during August–September, in 2009; from Morocco (Beni-Mellal region) in here they grow naturally. Sample were taken from 60 pods and stored at ambient temperature.

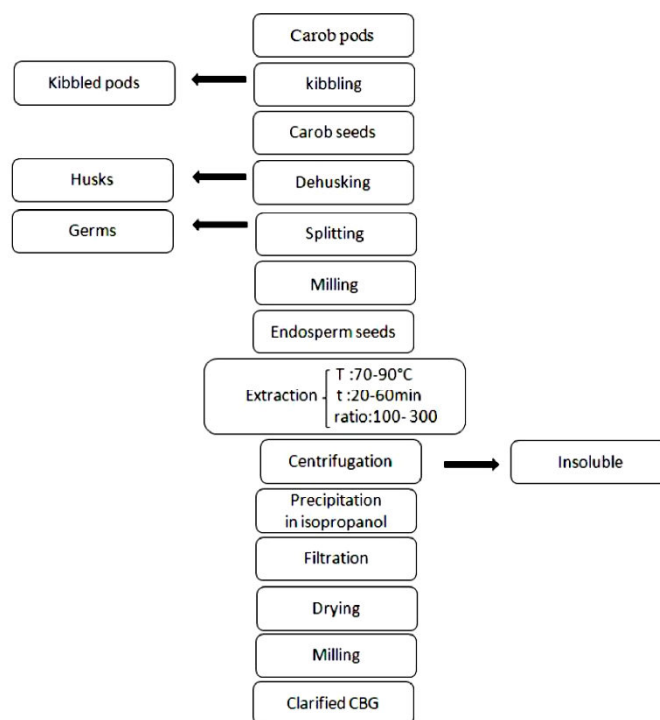


Figure 1. Extraction process of carob bean gum.

2.2. Extraction procedure

Figure 1 show the extraction and purification processes used in this work to obtain the purified CBG. The seeds are dehusked by treating the kernels with thermal mechanical treatments, followed by milling and screening of the peeled seeds to obtain the endosperm (native carob bean gum). The pretreated dry powder of crude carob bean gum was extracted with distilled water (ratio of water to endosperm of seeds ranging from (100 to 300), while the temperature of the water bath ranged from (70°C to 90°C), for a given time (extraction time ranging from 20 to 60 min).

The solution and the solid-phase were separated by centrifugation at (21875rpm, 1h). The Carob Bean Gum is precipitated with one volume excess of isopropanol. The white fibrous precipitate formed was collected by filtration with screen 45µm, and washed twice with isopropanol and with acetone. After drying under vacuum overnight at 30°C, the precipitate was ground to a fine powder.

2.3 Experimental design

The extraction parameters were optimized using RSM (Myers & Montgomery, 1995). The central composite design (CCD) was employed in this regard. The range and center point values of three independent variables presented in Table 1 were based on the results of preliminary experiments and on the results of other authors (Bouzouita et al. 2007; Pollard et al. 2010).

Table 1. Independent variables and their levels used for central composite rotatable design

	-1.68	-1	0	1	1.68
Extraction temperature °C	63.18	70	80	90	96.81
Extraction time (min)	6.36	20	40	60	73.63
Ratio of water to endosperm of seeds	31.82	100	200	300	368.17

CCD in the experimental design consists of eight factorial points, six axial points and six replicates of the central point (Table 2). Extraction temperature (X_1), extraction time (X_2) and ratio of water to endosperm of seeds (X_3)

were chosen for independent variables. Yield of polysaccharides was selected as the response for the combination of the independent variables given in (Table 2). Experimental runs were randomized to minimize the effects of unexpected variability in the observed responses.

The variables were coded according to the equation:

$$x_i = (X_i - X_0) / \Delta X \quad (1)$$

Where is the (dimensionless) coded value of the variable X_i , X_0 is the value of X_i at the centre point, and ΔX is the step change. Table 3 shows the actual design of experiments. The behavior of the system was explained by the following second degree polynomial equation:

$$Y = A_0 + \sum_{i=1}^3 A_i X_i + \sum_{i=1}^3 A_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 A_{ij} X_i X_j \quad (2)$$

2.4. Statistical analyses

Analysis of the experimental design and calculation of predicted data were carried out using NEMRODW Software to estimate the response of the independent variables. Subsequently, three additional confirmation experiments were conducted to verify the validity of the statistical experimental strategies.

3. Result and discussion

3.1. Preliminary study

Single-factor experimental designs (extracting temperature, extracting times, and ratio of water to endosperm of seeds ratio) were carried out before RSM experiments, in order to determine the experimental fields.

3.1.1. Temperature

To investigate the effect of extracting temperature on the yield of carob been gum, extraction process was carried out using different extraction temperature of 60, 70, 80, 90 and 100°C, while other extracting parameters were fitted as following: extracting time 40 min and extracting ratio of water to endosperm of seeds 200. As shown in Figure 2, there was an increasing trend in the yield of carob gum from 60 to 90°C. This tendency was in agreement with other reports in extracting polysaccharides (Vinogradov et al. 2003).

The maximum yield (68.7%) of polysaccharides was observed when extraction temperature was 95°C; the effect is not significant when extracting temperature is higher than 90°C. Therefore, 80°C was selected as the centre point of extracting temperature in the RSM experiments as higher temperature will bring about the energy waste and cost increase for extraction process.

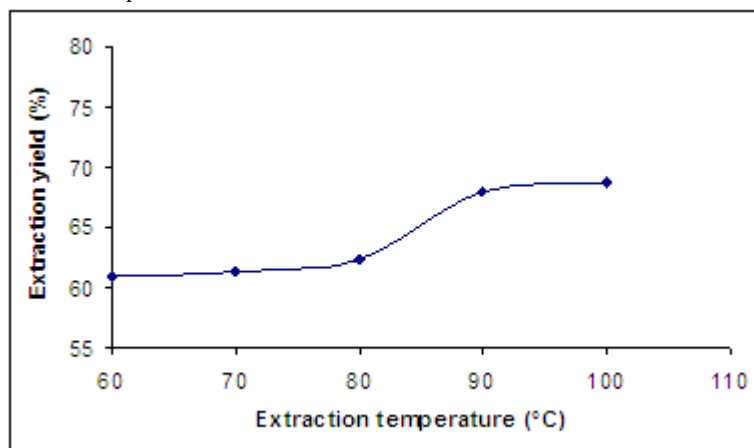


Figure 2. Effect of extraction temperature on extraction yield (Time = 40 min; Ratio of water to endosperm of seeds = 200).

3.1.2. Time

Extraction time is another factor that would influence the extraction efficiency and selectivity of the fluid. It was reported that a long extraction time also presents a positive effect on the yield of polysaccharides (Hou & Chen 2008). Extraction was carried out at different time conditions (10 to 70 min) while other extraction parameters were fixed at temperature = 80 °C and ratio of water to endosperm of seeds = 200.

The effect of different time on extraction yield of gum polysaccharides (CBG) is shown in Figure 3. When extraction time varied from 10 to 40 min, the variance of extraction yield was relatively rapid, and CBG yield reached a maximum at 40-60 min, and then became stable as the extraction proceeded. This indicated that 60 min was sufficient to obtain maximum yield of CBG extraction.

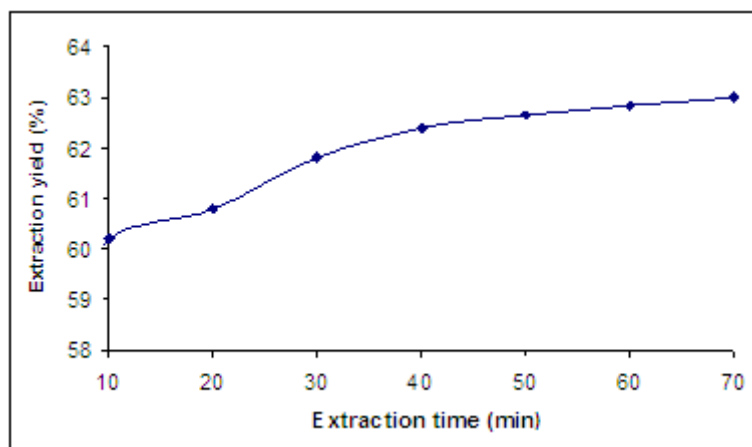


Figure 3. Effect of extraction time on extraction yield (Temperature = 80°C; Ratio of water to raw material = 200).

3.1.3. Ratio of water to endosperm of seeds

The effect of different ratio of water to endosperm of seeds on extraction yield of polysaccharides is shown in Figure 4. The extraction was carried out with ratios which vary between 50 and 350 under the following conditions of extraction: temperature = 80°C and Time = 40 min.

Figure 4 shows that the CBG yield increased significantly from 56.3% to 63.9% as the ratio of water to the endosperm of seeds increased from 50 to 350; this is due to the increase of the driving force for the mass transfer of polysaccharides (Bendahou et al. 2007). However, when the ratio continued to increase, the extraction yields no longer changed.

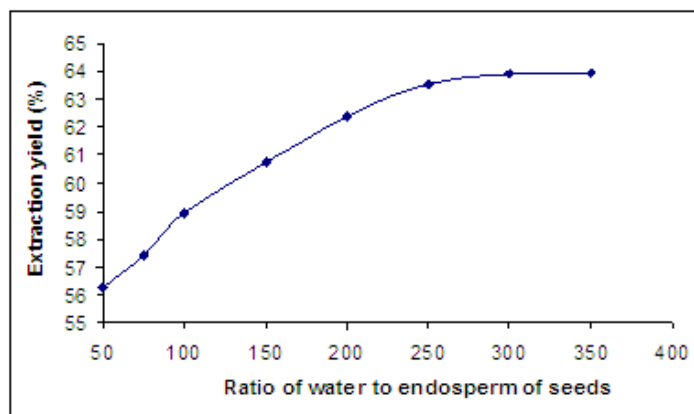


Figure 4. Effect of ratio of water to endosperm of seeds on extraction yield (Temperature = 80 °C; Time = 40 min).

3.2. Predicted model and statistical analysis

Table 3 shows the process variables and experimental data. The results of the analysis of variance, goodness-of-fit and the adequacy of the models are summarized. The percentage yield ranged from 54.3% to 69.1%. The maximum value was found at the extraction temperature 96.82°C, extraction time 40 min and ratio of water to endosperm of seeds 200. The application of RSM offers, based on parameter estimates, an empirical relationship between the response variable (extraction yield of gum) and the test variables under consideration. By applying multiple regression analysis on the experimental data, the response variable and the test variables are related by the following second-order polynomial equation (3):

$$Y = 62.39 + 2.09 * X_1 + 0.87 * X_2 + 2.68 * X_3 - 0.87 * X_1 * X_2 - 1.30 * X_1 * X_3 + 0.075 * X_2 * X_3 + 1.08 * X_1 * X_1 - 0.32 * X_2 * X_2 - 0.89 * X_3 * X_3$$

Where X_1 , X_2 and X_3 were the coded values of the test variables: extracting temperature (°C), extracting time (min) and ratio of water to endosperm of seeds, respectively.

Table 2. The central composite experimental design (in actual level of three variables) employed for extraction of CBG.

Run	Temperature (X1)	Time (X2)	Ratio of water to endosperm of seeds (X3)	* CBG yield (%)	
				Experimental	Predicted
1	70	20	100	54.3	54.3
2	90	20	100	63.1	62.5
3	70	60	100	57.8	57.9
4	90	60	100	62.6	63.1
5	70	20	300	62.4	61.9
6	90	20	300	65.5	65.4
7	70	60	300	65.7	65.4
8	90	60	300	65.8	65.8
9	63.18	40	200	62.1	62.4
10	96.81	40	200	69.1	69.1
11	80	6.36	200	60.1	58.9
12	80	73.63	200	63.2	62.4
13	80	40	31.82	55.6	55.6
14	80	40	368.17	64.5	64.4
15	80	40	200	62.4	62.4
16	80	40	200	62.5	62.4
17	80	40	200	62.5	62.4
18	80	40	200	62.0	62.4
19	80	40	200	62.5	62.4
20	80	40	200	62.4	62.4

* % per report has one grams of crude CBG.

The statistical significance of regression equation was checked by F-test, and the analysis of variance (ANOVA) for response surface quadratic polynomial model was done by software Nemrodw. The ANOVA of quadratic regression model demonstrated that the model was highly significant. And the Fisher's F-test had a very high model F-value (396.72) and a very low P-value ($P < 0.0001$). The value of R^2_{Adj} (0.9947) for Eq. (3) is reasonably close to 1, and indicates a high degree of correlation between the observed and predicted values. A very low value of coefficient of the variation (C.V.) (0.40 %) clearly indicated a very high degree of precision and a good deal of reliability of the experimental values. The lack-of-fit measures the failure of the model to represent the data in the experimental domain at points which are not included in the regression. The F-value (2.28) and P-value (0.1938) of lack-of-fit implied the lack-of-fit was not significant relative to the pure error. It indicates that the model equation is adequate for predicting the yield of carob gum under any combination of values of the variables. The lack-of-fit measures the failure of the model to represent the data in the experimental domain at points which are not included in the regression. The coefficient estimates of model equation, along with the corresponding P-values, were presented in Table 3. The P-values are used as a tool to check the significance of each coefficient, which also indicate the interaction strength between each independent variable. Smaller the P-value is, more significant the corresponding coefficient is (Muralidhar et al. 2001). When value of "probability > F" is less than 0.05. It can be seen from this table that the linear coefficients (X_1 , X_2 , X_3), a quadratic term coefficient (X_1^2 , X_2^2 , X_3^2) and cross product coefficients ($X_1 * X_2$, $X_1 * X_3$) were significant, with very small P values ($P < 0.01$). The other term ($X_2 * X_3$) are not significant ($P > 0.05$).

Table 3. Test of significance for regression coefficients

Effect	Coefficient estimate	Standard error	F-value	P value
X_1	2.0921	0.0672	968.8117	< 0.0001
X_2	0.8650	0.0672	165.6162	< 0.0001
X_3	2.6776	0.0672	1586.8752	< 0.0001
$X_1 * X_1$	1.0768	0.0654	99.2654	< 0.0001
$X_2 * X_2$	-0.3197	0.0654	219.1133	0.0006
$X_3 * X_3$	-0.8853	0.0654	0.7292	< 0.0001
$X_1 * X_2$	-0.8750	0.0878	270.8269	< 0.0001
$X_1 * X_3$	-1.3000	0.0878	23.8719	< 0.0001
$X_2 * X_3$	0.0750	0.0878	183.0890	0.4131

3.3. Response surface plot

The 3D response surfaces are the graphical representations of regression equation. They provide a method to visualize the relationship between responses and experimental levels of each variable and the type of interactions between two test variables. In the present study, the effects of the three factors as well as their interactive effects on the extraction rate are shown in figure 5(a), figure 5(b).

Figure 5a denotes the three dimensional surfaces plots of effect of extraction temperature (X_1) and the time of extraction (X_2) on response. As can be seen, enhancing the extraction temperature (X_1) from 70 to 90°C could increase the yield of CBG. Also, this increase is more significant on the yield of CBG when the time of extraction (X_2) is minimal. In the same way, the increase in the time of extraction from 20 to 60 min increases the yield of CBG significantly when temperature of extraction (X_1) is minimal.

Figure 5b shows the effect of extraction temperature (X_1) and the ratio of water to endosperm of seeds (X_3) on the yield of polysaccharides. It was observed that yield of CBG increased with the increase in the temperature of extraction (X_1) from 70 to 90°C. Also, this increase is more significant on the yield of extraction when the ratio of water to endosperm of seeds (X_3) is minimal. In the same way, the increase in the ratio of water to endosperm of seeds (X_3) from 100 to 300 increases the yield of CBG significantly when temperature of extraction (X_1) is minimal.

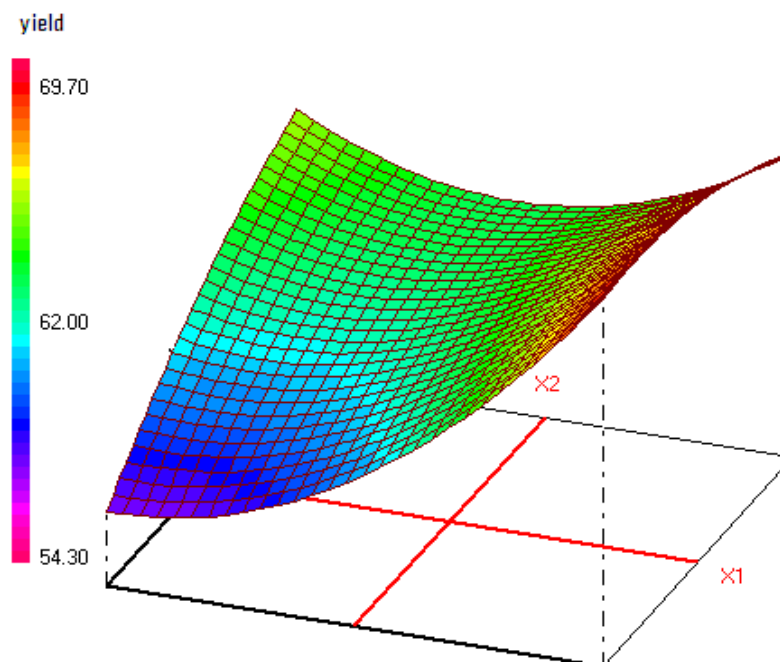


Figure 5a. Response surface plots and showing the effect of extracting temperature (X_1) and time (X_2) on the yield of CBG.

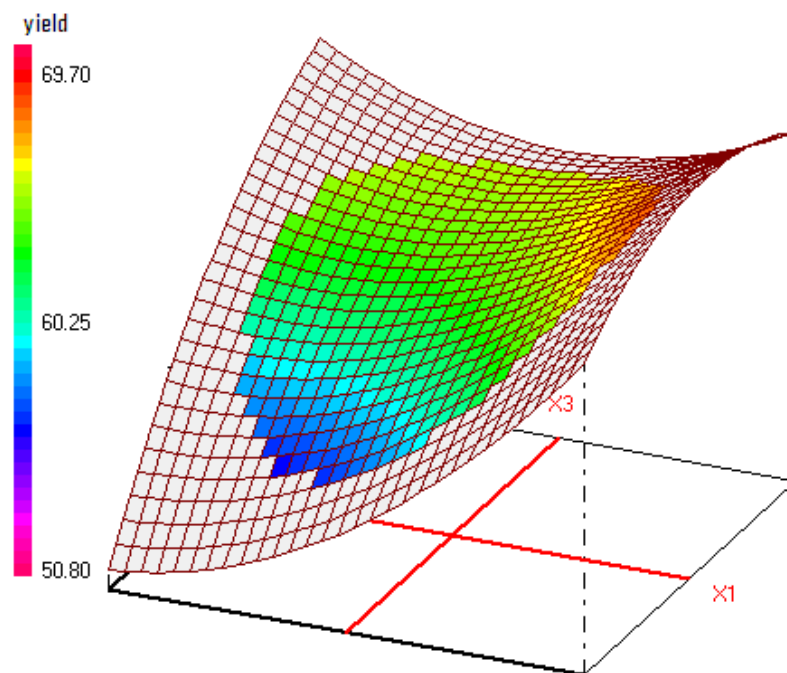


Figure 5b. Response surface plots and showing the effect of extracting temperature (X_1) and ratio of water to endosperm of seeds (X_3) on the yield of CBG.

3.4. Optimization of extracting parameters and validation of the model

The optimum conditions were: Temperature 97°C, time 36 min and water to endosperm of seeds ratio at (197:1). To ensure the predicted result was not biased toward the practical value, experimental rechecking was performed using this deduced optimal condition. A mean value of 69.7 ± 1.03 ($N = 3$), obtained from real experiments, demonstrated the validation of the RSM model. The good correlation between these results confirmed that the response model was adequate for reflecting the expected optimization

4. Conclusions

The performance of the extraction of carob bean gum was studied with a statistical method based on the response surface methodology in order to identify and quantify the variables which may maximize the yield. The three variables chosen, namely extraction Temperature, extraction time, and ratio of water to endosperm of seeds ratio all have a positive influence on the yield of polysaccharides using the extraction method. The optimal conditions obtained by RSM for production of CBG include the following parameters: extraction temperature 97°C, extraction time 36 min, and ratio of water to endosperm of seeds 197.

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